Stability indicating HPTLC method for the determination of pregabalin in bulk and pharmaceutical dosage forms

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ABSTRACT

The present study focuses on the development, validation and force degradation studies of Pregabalin using high performance thin layer chromatography (HPTLC). The method was developed by optimizing the mobile phase with butanol: methanol: water : glacial acetic acid with a ratio of 8: 1: 1: 0.1. A good correlation coefficient was observed in the concentration range of 200 ng/spot- 1000 ng/spot. The developed method was validated for intraday and inter-day precision and low percentage RSD values of 1.65 and 1.73 % respectively, were observed indicating the suitability of the developed method. The proposed method was also used for assay of capsules. Recovery studies were performed and it was found that the method is accurate, sensitive and selective for the analysis of the drug. Force degradation studies were carried out using ICH tripartite guidelines and found that the drug is stable in neutral, acidic, thermal and photolytic conditions. However, it showed a degradation of approximately 54.99 % on exposure to alkaline stress conditions and 55.13 % on exposure to the oxidative stress conditions.   © 2013 Trade Science Inc. - INDIA

INTRODUCTION

Purity, quality, efficacy are the important features for the stability of the bulk drugs and pharmaceutical dosage forms. International Conference on Harmonization (ICH) published guidelines on Analytical Method Validation (Q2B), Stability testing of new drug substances and products, (Q1A (R2)) and Photostability testing of new drug substances and products (Q1B), are used as a mandidatory steps for checking the quality of the bulk drugs and pharmaceutical dosage forms in Quality Control laboratories. Validation tells how good the method is specifically whether it is good for the intended application or not. The method validation is a continuous process and the main goal is that the analytical data is in confidence for the intended use. Thus the method validation is the major concern in the activity of analytical chemistry laboratories. The information obtained from stress testing can be used for recommending storage conditions, formulation and development of dosage forms and their appropriate packaging design[1].

Pregabalin, (S)-3-(Aminomethyl)-5-methylhexanoic acid is a gabapentin-like agent that has been approved in U.S. for treatment of painful diabetic neuropathy (PDN), post herpetic neuralgia (PHN), partial seizures (PS), and fibromyalgia (FBM)[2]. Pregabalin binds to the alpha-2-delta (A2D) receptors of an auxiliary subunit associated with voltage-gated calcium channels in central nervous

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system tissues, and thereby inhibits influx of calcium and release of glutamate, norepinephrine, substance P, and other neurotransmitters. It is white to off-white crystalline solid with PKa values of 4.2 and 10.6[3]. It is freely soluble in water and both basic and acidic aqueous solutions. Pregabalin undergoes negligible metabolism in humans with mean renal clearance was estimated to be 67.0 to 80.9 ml/min in young healthy subjects. Till date, various analytical methods have been reported for the determination of pregabalin in bulk and pharmaceutical preparations after derivatization viz., enantioselective chromatographic determination after precolumn derivatization using Marfey's reagent[4], spectrophotometric determination using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and 7,7,8,8-tetracyanoquinodimethane (TCNQ)[5] and spectroflourimetric determination using 7-chloro-4-nitrobenzofurazon (NBD-Cl)[6]. Various methods have also been established for enantiomeric separation of the drug viz., HPLC using tendon mass spectrophotometry[7], the drug can also be estimated in serum and plasma via validated sensitive HPLC methods[8,9]. Stability parameters of Pregabalin has not been reported anywhere in the literature. Hence an attempt has been made to develop and validate a HPTLC method for analyzing the drug using ICH guidelines.

EXPERIMENTAL

Apparatus

Stability studies were carried out on the HPTLC, CAMAG (Switzerland) with CAMAG auto sampler, CAMAG TLC scanner and WINCAT software.

Reagents

Pregabalin was gifted by Ranbaxy Ltd, India. All reagents used were of analytical grades. Butanol (Qualigens fine chemicals, India), methanol (Qualigens fine chemicals, India), glacial acetic acid (Merck limited, Mumbai, India).

Chromatography conditions

Chromatography was performed on 20 cm × 10 cm aluminium-backed plate coated with 0.2 mm layer of silica gel 60 F_{254} (E.Merck, Germany). Samples were applied to the plates as 6 mm bands, 10 mm apart, by means of a Camag (Switzerland) automatic sample ap-

plicator fitted with a camag microliter syringe. A constant application rate of 150 nL/s was used. Linear ascending development of the plates to a distance at 80 mm was performed with Butanol: Methanol: Water: Glacial acetic acid, 8:1:1:0.1 v/v, as mobile phase in a twin-trough glass chamber previously saturated with mobile phase for 10 minutes at room temperature (25°C). After development the plate was scanned at 390 nm by means of a Camag TLC scanner in absorbance mode using deuterium lamp. The slit dimensions were 5 mm × 0.45 mm and the scan speed was 20 mm/s.

Method development

The method has been developed by optimizing the mobile phase for the determination of pregabalin in bulk drug and in pharmaceutical preparation using different solvents like chloroform, acetonitrile, glacial acetic acid, butanol, ethanol, methanol, and water. The suitability of the mobile phase was decided based on sensitivity of the assay, the time required for the analysis and the use of readily available solvents.

Method validation

(a) Linearity

Stock solution of 100 µg/mL was prepared in water and different volumes of dilutions i.e.,2, 4, 6, 8, and 10 µL was applied on the 20 cm ×10 cm pre-coated TLC plate in triplicate using Camag (Switzerland) automatic sample applicator fitted with a Camag micrilitre syringe. The plate is allowed to develop in twim-trough chamber having Butanol: Methanol: Water: Glacial acetic acid, 8:1:1:0.1 v/v as mobile phase for 45 minutes at room temperature.

(b) Precision

Precision was determined by two methods i.e., repeatability and intermediate precision according to the ICH guidelines.

(A) Repeatability

Repeatability of the developed method was investigated by interday variation i.e., determination of the three different concentration in triplicate in three consecutive days.

(B) Intermediate precision

Intermediate precision of the developed method was investigated by intraday precision i.e., determination of

the three concentration in triplicate in a single day.

Assay procedure for the marketed preparations

For the preparation of the sample solution ten capsules were weight and powder, then a portion of powder equivalent to 10 mg of PGB were transferred into 100 mL of volumetric flask and volume was made upto 100 mL with distilled water. Ten microlitre of the above sample solution i.e., 1 µg/mL is spotted on pre-coated TLC plate using a Camag (Swizerland) automatic sample applicator fitted with a Camag microlitre syringe and developed in twin-trough chamber having Butanol: Methanol: Water: Glacial acetic acid, 8:1:1:0.1 v/v as mobile phase for 45 minutes at room temperature.

Recovery

The recovery study was performed to check the accuracy of the developed method. For the recovery studies three dilutions equivalent to 30, 100, 150 µg/mL were prepared by spiking 10, 150 and 250 µg/mL with 50 µg/mL respectively, similar concentrations were prepared form stock. 10 µL/spot of each dilution were spotted on the pre-coated TLC plate using a Camag (Switzerland) automatic sample applicator fitted with a Camag microlitre syringe.

Robustness

Robustness of the developed method was seen by deliberately changing the mobile phase concentration, time of developing the plate and activation of plate and the effect on the results was examined.

Limit of detection (LOD) and limit of quantitation (LOQ)

The detection limit and quantitation limit were calculated by the following formula

LOD=3.3σ/S

LOQ=10σ/S

Where,

"σ" is the standard deviation of the response and "S" is the slope of the calibration curve.

Force degradation studies

Force degradation studies were carried out by neutral hydrolysis, acid hydrolysis, alkaline hydrolysis, oxidation, thermal degradation and photo stability studies according to the ICH guidelines and Guidance for the Conduct of Stress Testing of Drug Substances[10].

Hydrolysis studies

(a) Neutral hydrolysis

Samples of the drug at a concentration of 100 µg/ml were prepared for each step with distilled water and were tested both before and after exposure to the respective stress conditions (25°, 40° and reflux respectively). These samples were then analysed by HPTLC system. The area under the curve at 0-point was taken as 100% of the drug for each set. Therefore the amount of drug remaining after exposure to the stressed conditions was calculated corresponding to the values at 0 point.

(b) Acid and alkaline hydrolysis

Samples of the drug at a concentration of 100 µg/ml were prepared for each step with various normalities of HCl and NaOH (0.1N, 1N, 2N and 5N), and were tested both before and after exposure to these conditions. These samples were then analysed by HPTLC system. The area under the curve at 0-point was taken as 100% of the drug for each set. Therefore the amount of drug remaining in all the treated samples after exposure to the stressed conditions was calculated corresponding to the values at 0 point (untreated sample).

Oxidation

Samples of the drug at a concentration of 100 µg/ml were prepared with 1% of H_2O_2, and were tested both before and after exposure to varying strengths of H_2O_2 viz., 30%, 10%, 3% and 1% for 1 day, 6 h, 3 h and 30 minutes respectively. These samples were then analyzed by HPTLC system. The area under the curve at 0-point was taken as 100% of the drug for each set. Therefore the amount of drug remaining after exposure to the stressed conditions was calculated corresponding to the values at 0 point.

Photo-degradation

(a) Instrumentation

The photostability studies were carried out in the stability chamber (KBWF 240, WTB Binder, Germany) equipped with light source (option 2) as specified in the ICH guidelines Q1B and maintained at 40ºC and 75% RH. The samples were withdrawn in time intervals for 3 months.

The following study was carried out on the drug

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both in solid and solution forms. The samples for stress studies were kept in stability chamber (KBWF 240, WTB Binder, Germany) equipped with light source (option 2) as specified in the ICH guidelines Q1B and maintained at 40ºC and 75% RH. Two sets of the test samples of the drug were prepared in solid and solution state. Each set comprised of a wrapped and unwrapped sample. Protected samples (e.g., wrapped in aluminum foil) were used as dark controls to evaluate the contribution of thermally induced change to the total observed change, these were also placed alongside the unwrapped samples. Samples of the drug at a concentration of 100 µg/ml were prepared for each step with water in triplicate, and were analyzed before and after exposure to the stress conditions by HPTLC system. The area under the curve at 0-point was taken as 100% of the drug for each set. Therefore the amount of drug remaining after exposure to the stressed conditions was calculated corresponding to the values at 0-point. The samples were kept there for a period of three months. Analysis of both the solid and solution samples was performed at regular intervals of 0-day, 15th-day, and 90th-day. The samples were then analyzed by HPTLC.

Thermal degradation

Thermal degradation study is performed by keeping the powder at 70ºC for 15 days. The standard and stressed sample was analyzed by dissolving 100 mg in 100 mL of water and analyzed using HPTLC.

RESULTS AND DISCUSSION

Optimization of the method

An HPTLC method was optimized in order to develop an stability-indicating assay method. The drug reference standard was spotted on the TLC plate and developed using an optimized mobile phase shown in TABLE 1 and analyzed using HPTLC CAMAG TLC scanner. A well defined spots were observed when the chamber is saturated with butanol: methanol: water: glacial acetic acid, 8: 1:1: 0.1 v/v as mobile phase for 45 minutes at room temperature. The R_f values of 0.36±0.05 were obtained.

TABLE 1 : Optimization of the mobile phase

S.No.	Mobile Phase Used		Remarks
	Solvents	**Ratios**	
1	Ethylacetate: methanol: water: dimethylsulfoxide	10: 6: 4: 1	No Spots were obtained
2	Butanol: Ethanol: Water	9: 7: 4	Tailing
3	Butanol: Glacial acidic acid: Water	9.3: 0.25: 0.5	Tailing
4	Chloroform: Ethanol: Water	8: 1: 1	No Spots were Obtained
5	Butanol: Methanol: Water	9: 7: 4	Tailing
6	Butanol: Methanol: Water	8: 1: 1	No Tailing
7	Butanol: Methanol: Water: Glacial acidic acid	8: 1: 1: 0.1	Well defined Spots were obtained

Method validation

(a) Linearity

Good linearity relationship was found between the peak areas at the concentration range of 200 ng/spot – 1000 ng/spot. The regression equation was found to be y= 14.94x+5962 with r2 value of 0.996 as shown in Figure 1. Scanning profile of TLC chromatogram of 200, 400, 600, 800 and 1000 ng/spot of pregabalin and Chromatography of standard pregabalin (1000 ng/spot) was shown in Figure 2 and Figure 3.

(b) Precision

The results of repeatability and intermediate precision are shown in TABLE 2. The developed method was found to be precise as the % RSD was found to be less than two as recommended by ICH guidelines.
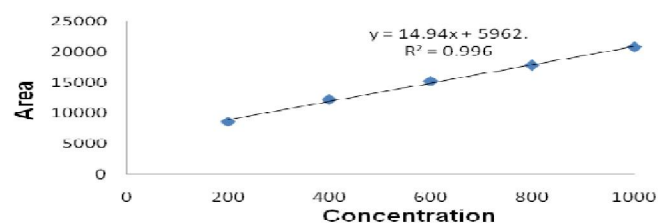

$y = 14.94x + 5962.$
$R^2 = 0.996$

Figure 1 : Linearity plot of pregabalin

Assay for marketed preparations

The developed method was tested for the marketed preparations and found that the method is accurate and precision as it indicated an excellent percentage recov-

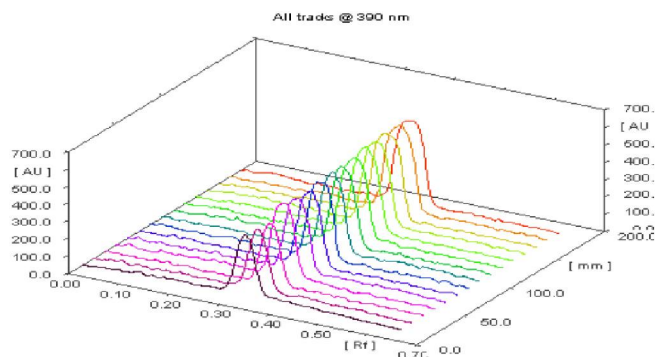ery of 102.66% with no interference of the excipients present in the capsules.

Figure 2 : Scanning profile of TLC chromatograms of 200. 400, 600, 800 and 1000 ng/spot of pregabalin
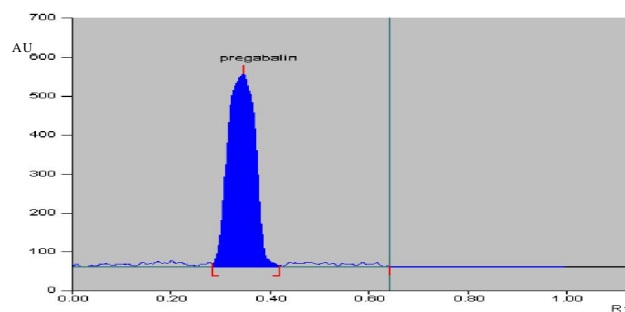

Figure 3 : Chromatogram of standard pregabalin (1000 ng/ spot) with Rf value 0.36±0.05, mobile phase butanol: methanol: water: glacial acidic acid (8:1:1:0.1)

TABLE 2 : Precision studies (n=3)

Concentration (ng/spot)	Repeatability		Intermediate	
	Mean± S.D	% RSD	Mean± S.D	% RSD
200	8521.2±129.05	1.51	9424±163.85	1.73
600	15148±243.34	1.59	15144.1±238	1.57
1000	19775±326.63	1.65	18321±110.06	0.60

(a) Recovery

Recovery studies was carried out for the developed HPTLC method and found that the average percentage of recovery was 104% which indicates good accuracy for the developed method.

(b) Robustness

Robustness of the developed method was tested by deliberately changing the mobile phase, development time and optimization time and found that the developed method is robust as there is no remarkable change in the area and percentage RSD as shown in TABLE 3.

(c) Force degradation studies

(A) Neutral hydrolysis

The force degradation studies in neutral conditions were carried out using ICH guidelines and the results of the stress studies were illustrated in TABLE 4. The drug peak showed decreased in area after 24 hours of reflux, which clearly indicates that the drug is stable in neutral conditions.

TABLE 3: Robustness studies

S.No.	Parameters	Concentration (ng/spot)	Area (ng/spot)	% RSD
1	Development of TLC plate for one hour	200	8856.4	1.9
		600	16250.2	1.9
		1000	19658.9	1.9
2	Development of TLC plate for thirty minutes	200	8766.6	0.6
		600	15768.9	0.5
		1000	20225.8	1.0
3	Development of TLC plate in 15 ml of mobile phase	200	8965.9	0.5
		600	15887.9	1.6
		1000	20773.8	1.5

TABLE 4: Extent of decomposition observed on exposure to neutral hydrolytic stress conditions

S.No	Time of Exposure	Temperature	Amount Remaining	Extent of Decomposition
1	2 h	25ºC	101.1%	None
2	8 h	40ºC	100%	None
3	12 h	Refluxing	82%	None
4	24 h	Refluxing	22.7%	Sufficient

(B) Acidic and alkaline hydrolysis

The stress studies were carried out in acidic conditions using ICH guidelines and the results were illustrated in TABLE 5. The drug peak did not show any decrease in the peak area even after 2 days of refluxing in 5N HCl, which indicated that the drug is highly stable in acidic conditions. However, the drug peak sufficient degradation in alkaline medium i.e. approximately 55% drug was left after exposure to 0.1 N NaOH for 8 h. The chromatograms of PGB before and after reflux in alkaline conditions are shown in Figure 4 and Figure 5.

(C) Oxidation

Peak area decreased sufficiently upto 54%, after oxidative stress conditions in 1% H_2O_2 for three hours. Chromatographic peak before and after stress conditions were shown in Figure 6 and Figure 7. Furthermore no extra peaks were observed in the chromato-

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gram, indicating the formation of non-chromophoric degradation products.

TABLE 5: Extent of decomposition observed on exposure to alkaline stress conditions

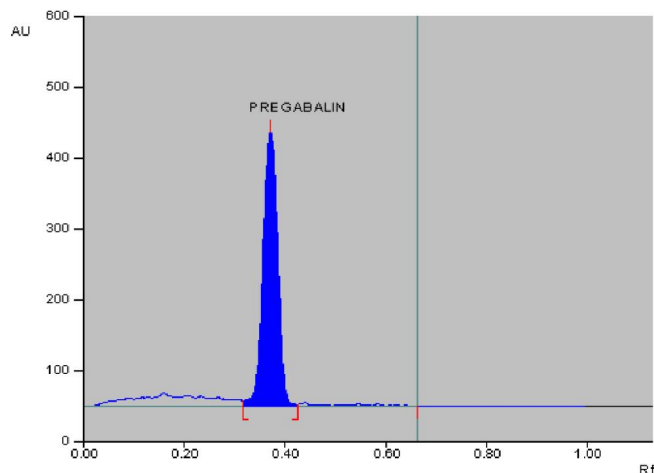| S. No.	Strength of Acid (HCl)	Time of Exposure	Temperature	Amount Remaining (%)	Extent of Decomposition
1	0.1N	8 hours	Refluxing	97.6	None
2	1N	12 hours	Refluxing	97.43	None
3	2N	1 day	Refluxing	98	None
4	5N	2 days	Refluxing	101	Not Sufficient

Figure 4 : Chromatogram of the drug before reflux in 0.1N NaOH
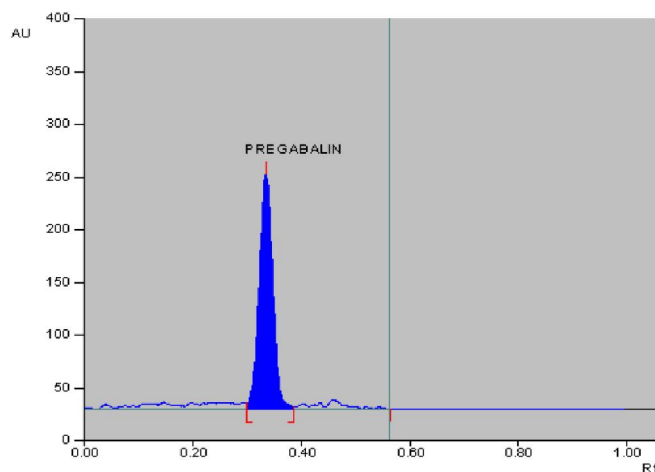

Figure 5 : Chromatogram of PGB after reflux for 8 hrs in 0.1 N NaOH

(D) Photostability studies

The photolytic studies were conducted on both the drug solution and powder drug sample. The stressed sample was analyzed by HPTLC after proper exposure and the result obtained are illustrated in TABLE 6. Peak area did not decrease sufficiently. From the re-

sults of the degradation studies it was observed that the drug was photostable in the solid and solution state. No sufficient degradation of drug was observed even after 90 days i.e., 3 months of exposure and there is no change in the color of the solution or powder.
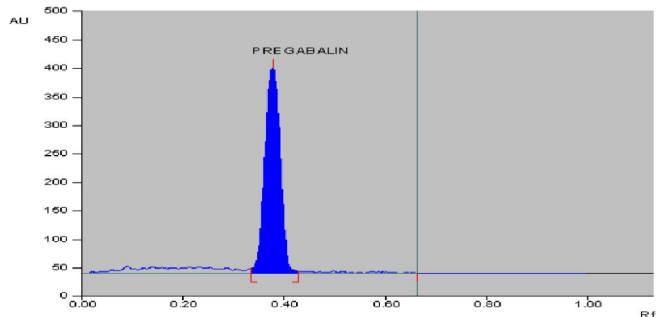

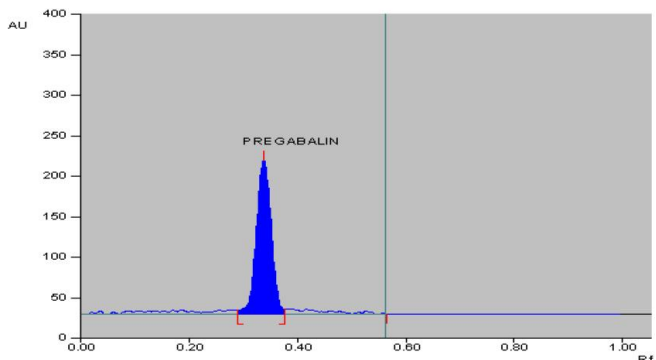**Figure 6 : Chromatogram of PGB before exposure to oxidative stress**

Figure 7 : Chromatogram of PGB after exposure to oxidative stress conditions (3 hrs with 1% H_2O_2) Photostability studies

TABLE 6: Extent of decomposition observed in the drug in solid state and in solution form kept under photo-degradation conditions

S. No	State of drug	State of Exposure	Time (days)	Amount Remaining (%)	Extent of decomposition
1	Solid		0	100	None
		Unwrapped	15	104.73±1.00	None
			90	102.53±0.99	Not sufficient
			0	100	None
		Wrapped	15	102.26±0.47	None
			90	101.12±0.02	Not sufficient
2	Solution		0	100	None
		unwrapped	15	100.19±0.19	None
			90	100.12±0.04	Not sufficient
			0	100	None
		Wrapped	15	104.25±0.05	None
			30	98.98±0.01	Not sufficient

(E) Thermal degradation

Thermal degradation studies were carried out on powder by keeping it in 75ºC for 15 days and it was

found that the peak areas did not decrease which indicating that the drug is thermally stable.

CONCLUSION

Thus the developed HPTLC method was found to be accurate and sensitive for the determination of the drug in both bulk as well as pharmaceutical formulations. The validated results show that the method has good linearity and % RSD values are found to be within the acceptable range. The overall inference drawn from above stability study is schematically indicated in TABLE 7.

TABLE 7: Overall inference of stress testing studies

S.No.	Stress Condition	Interpretation
1	Neutral hydrolysis	Stable
2	Acidic hydrolysis	Stable
3	Alkaline hydrolysis	Susceptible
4	Oxidation	Susceptible
5	Photo-degradation	Stable
6	Thermal degradation	Stable

ACKNOWLEDGEMENT

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